A dual stimuli responsive supramolecular gel provides insulin hydrolysis protection and redox-controlled release of actives

*Diego Navarro-Barreda, César A. Angulo-Pachón, Begoña Bedrina, Francisco Galindo and Juan F. Miravet**

D. Navarro-Barreda, Dr. C. A. Angulo-Pachón, B. Bedrina, Prof. F. Galindo and Prof. J. F. Miravet
Department of Inorganic and Organic Chemistry, University Jaume I, 12071 Castelló de la Plana, Spain
miravet@uji.es

Keywords: supramolecular gel, responsive gels, insulin hydrolysis, controlled release, disulfide gels

Two supramolecular hydrogelators containing a central disulfide moiety and terminal carboxylic acid groups are studied. On the one hand, the hydrogels are responsive to a reductive environment, which transforms the disulfide unit to the corresponding thiols. On the other hand, the hydrogels show pH response associated with the presence of carboxylic acid units. Gels are formed at pH below ca. 4 while at higher pH values, ionization of the gelators provokes gel disassembly.

The properties of the gel are exploited for the release, as a proof of concept, of Bromophenol Blue in the presence of the reducing species tris(2-carboxyethyl)phosphine hydrochloride (TCEP). Additionally, insulin is loaded into the hydrogels and protected from hydrolysis with simulated gastric fluid (SGF) containing pepsin. Quantitative release of unaltered insulin, checked with an ELISA colorimetric assay, is observed upon treatment with pH 7.4 buffer. This behavior would permit the use of the new hydrogels for oral insulin delivery.

1. Introduction

The development of smart, stimuli-responsive, materials is undoubtedly a major driving force in chemistry and materials science nowadays. As for soft organic materials, intelligent systems are associated with polymeric gels to a great deal.[1-2] For example, these materials can contract in response to electric fields, light or temperature changes, as revealed in several fundamental reports.[3-6] The potential biomedical application of responsive gels has been explored to a considerable extent. Temperature responsive gels were already used in the 1990s for protein and peptide delivery.[7] Polymeric gel networks permit to design biomedical devices that respond to stimuli such as temperature, pH, or the presence of different molecules such as glucose, glutathione or antigens, among others.[8] In particular, the use of polymeric gel matrices for drug delivery has been extensively studied and reviewed.[9-11]

Molecular (supramolecular) gels, unlike conventional gels formed by polymers, are constituted by low molecular weight molecules, which self-assemble into solvent percolating fibrillar networks. The interest in these soft supramolecular materials has grown exponentially in the last two decades.[12-14] In particular, molecular hydrogels are receiving much attention due to their potential biomedical applications.[15-16] Distinctive characteristics of supramolecular gels are their intrinsic reversibility and stimuli responsiveness. Due to supramolecular nature of the interactions, the fibrillar network can usually be reversibly assembled/disassembled by temperature changes or, upon proper design, by different stimuli such as saline effects, light, pH change, enzymes or different chemical species.[17-19] It has to be noted that stimuli responsiveness in polymeric gels is mostly associated with swelling/shrinking processes, but in molecular gels, the response to the stimulus results commonly in gel disassembly. This property makes supramolecular gels ideal for stimuli-triggered controlled release as shown, for example, in a recent review on this topic,[20] or for their application in regenerative medicine.[21]

The introduction of ionizable groups in the structure of molecular hydrogelators results in pH-sensitive gels. A relatively large number of pH-sensitive supramolecular gels have been described in the literature. Carboxylic acid moieties are common motifs in pH-responsive molecular gels,[22-24] but a variety of ionizable units have been incorporated such as phenol,[25] histidine,[26] triazole,[27] pyridine[28] or guanidinium,[29] among others. On the other hand, the presence of disulfide moieties in gels affords responsiveness to a reductive environment, like that generated in cells by the presence of glutathione. On this regard, quite a few polymeric gels have been reported containing disulfide linkages, which can be reduced to thiols by glutathione provoking the release of the gel cargo. For example, this approach has been demonstrated for the release of paclitaxel,[30] doxorubicin,[31-32] plasmid DNA,[33] and methotrexate.[34] However, only a reduced number of low molecular weight gelators with disulfide moieties have been described. The most notable case is dibenzoyl-L-cystine, a gelator reported in 1921[35] that was studied in detail in an illuminating paper from Menger et. al.[36] This gelator has been recently used for the preparation of transient supramolecular hydrogels based on the disulfide/thiol interconversion chemistry.[37] Cysteine is also a structural unit for other molecular gelators with disulfide-thiol chemistry,[38-42] but other building blocks have also been used like diphenyldisulfides[43] and commercially available cystamine.[44-45] Interestingly, disulfide reduction, commonly with glutathione,[41, 46] dithiothreitol[46-47] or tris(2-carboxyethyl)phosphine hydrochloride (TCEP)[38, 48] can result either in gel disassembly[39-40, 42-44] or assembly[38, 41, 45] depending on the system.

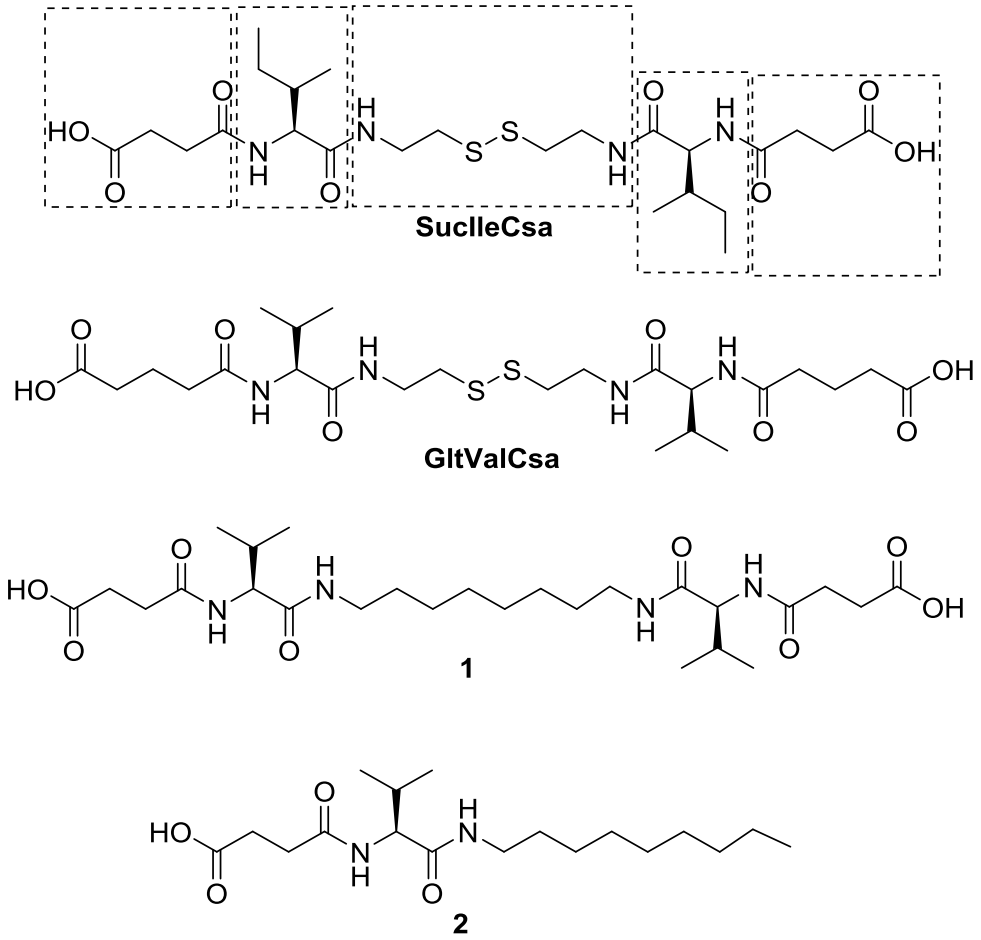
A notable application of polymeric gels is their use for protection of insulin aiming for its oral delivery. The first biological barrier against any orally administered proteins of therapeutic use, such as insulin, is the harsh acidic conditions inside the stomach together with the presence of gastric enzymes, resulting in rapid hydrolysis of peptides and proteins. Different polymeric hydrogels formed, for example, by poly(methacrylic acid) grafted with poly(ethylene glycol),[49] carboxymethylcellulose/poly(acrylic acid)[50] or food gums tragacanth[51] and

salecan[52] protected insulin towards hydrolysis in gastric fluids. Nanoparticles or microgels prepared from different biodegradable polymers such as chitosan or poly(lactic-co-glycolic acid), among many others, have tested for their use in oral insulin delivery.[53-55] However, up to our knowledge, supramolecular gels have not been tested for this purpose.

Here we report on a new supramolecular gelator containing both a redox-sensitive disulfide group and pH-responsive carboxylic acid unit. The responsiveness of the formed gels towards the disulfide reducing agent TCEP and pH are assayed. As a proof of concept, the release of the entrapped dye Bromophenol Blue is studied. Additionally, the protection of insulin towards hydrolysis under simulated gastric fluid and later discharge of the protein at simulated intestinal pH is also described.

2. Results and discussion

In previous work, we have described a few amphiphilic and bolaamphiphilic hydrogelators constituted by amino acid units which are N-acylated with succinic anhydride and present alkyl amides formed from the carboxylic acid function (see at Scheme 1 compounds **1** and **2** as an example).[56-58] Based on that structure and to introduce reduction responsive units, two new isomeric bolaform molecular gelators were built from amino acids L-isoleucine or L-valine, cystamine and succinic or glutaric anhydride (Scheme 1). The preparation of compounds **SucIleCsa** and **GltValCsa** was found to be efficient and straightforward. Acylation of commercially available cystamine with the corresponding C-activated amino acid was followed by N-acylation of the amino acid residue with the corresponding anhydride (see SI, for details).



Scheme 1. Structure of the studied compounds **SucIleCsa**, **GltValCsa,** and related compounds **1** and **2**.

SucIleCsa and **GltValCsa** are pH-sensitive hydrogelators which form gels only in their neutral form at acidic pH. Potentiometric titration of **SucIleCsa** revealed apparent pK_a values of 4.2 and 5.1 for the first and second deprotonation, respectively. Similar results were obtained for **GltValCsa** with apparent pKa values of 4.3 and 4.6. Apparent pK_a values englobe the thermodynamic balance associated with acid-base and aggregation equilibria.[22, 59] Figure 1 shows the calculated species distribution species diagram for **SucIleCsa**, which reveals that neutral species, capable of hydrogelation, are predominant in solution only below pH = 4. At higher pH values, the ionic species are water-soluble, precluding gel formation.

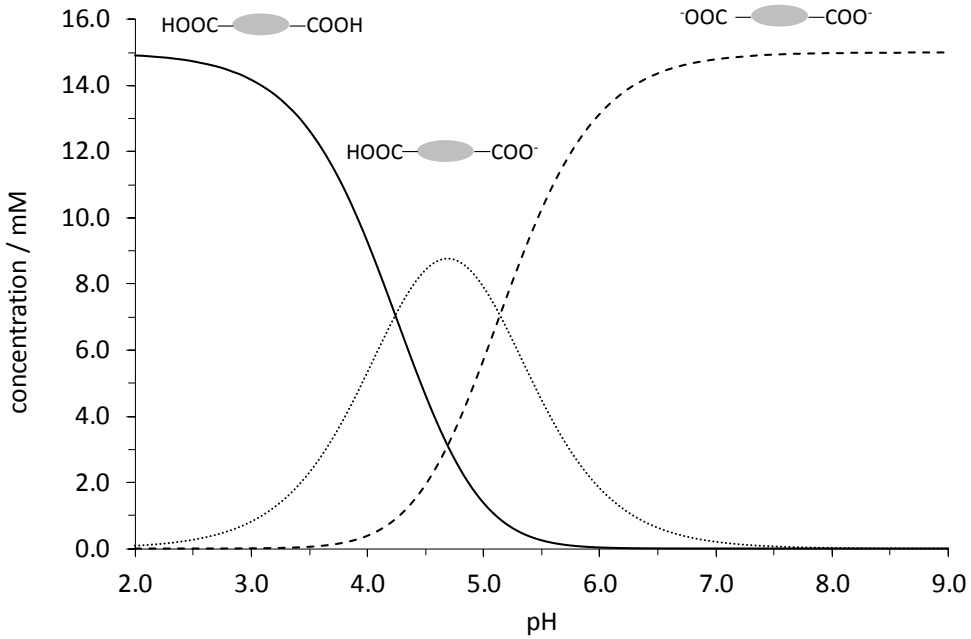


Figure 1. Calculated species distribution diagram for **SucIleCsa** (15 mM).

The study of the hydrogelation capabilities was performed at room temperature by addition of 0.1 M HCl to a 0.1 M NaOH solution of the gelator. Opaque hydrogels were formed with minimum gelation concentration values of 15 mM, determined by vial inversion methodology, for both **SucIleCsa** and **GltValCsa**. Thermal stability was tested with the vial inversion test for 18 mM gels, as those used in the experiments reported below. A T_{gel} value of 80 ± 5 ºC was measured for **GltValCsa** while the gel formed by **SucIleCsa** was not diassembled even at 95 ºC. Rheology studies confirmed the gel-like nature of the soft materials formed, being the elastic modulus, G', considerably higher than the viscous modulus, G''(see Figure S1). For gels with a concentration of 18 mM, the viscous moduli showed significative differences between **SucIleCsa** (24,500 Pa) and **GltValCsa** (2,500 Pa). Probably the different morphology of the self-assembled networks, discussed in the next lines, could account for such differences. Transmission electron microscopy (TEM) images of the corresponding xerogels revealed an entanglement of self-assembled fibrillar objects observed commonly in molecular gels (Figure 2 and Figure S12). Noticeably, the fibers formed by **SucIleCsa** are thinner and more curved than those found for **GltValCsa** which are flatter and straighter. Additionally, the addition of

the fluorescent dye Nile red, that is adsorbed on the fibers, permitted to obtain images by confocal scanning laser microscopy (CSLM) of the fibrillar structure, without removal of the solvent (Figure 2 and Figure S13, S14).

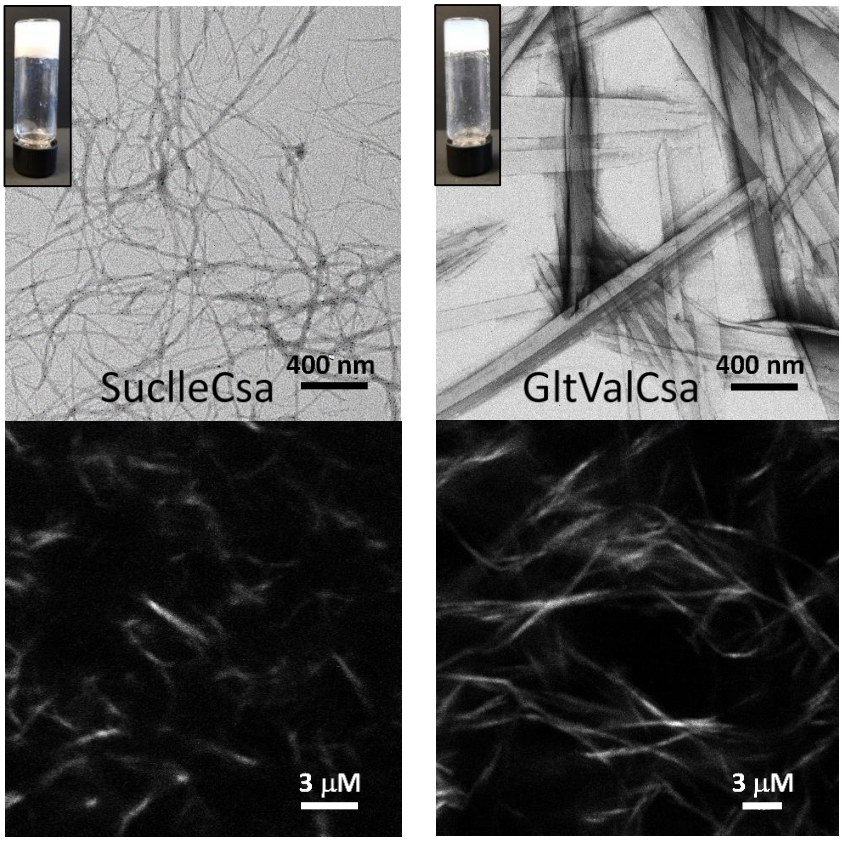


Figure 2. Top: TEM images of the xerogels. Bottom: CSLM images of the hydrogels stained with Nile red.

Gels of **SucIleCsa** and **GltValCsa** (18 mM for both of them) were treated with TCEP at 37.4 °C to evaluate their stability to a reducing environment. Gel disassembly could be monitored by NMR taking advantage of the fact that the fibrillar network is NMR silent,[60] but the reduced thiols obtained are NMR visible (see Figure 3 for data on **SucIleCsa**, similar results were obtained for **GltValCsa**, shown at Figure S4). The NMR integration data collected at different time intervals (see Figure S6, S7) were fitted to first order kinetics. It has to be noted that the fibrillar network is solid-like and therefore does not intervene in the kinetic constant. Additionally, the amount of free gelator in equilibrium with the gel network is constant because of the solubility equilibrium present.[60] Half-life times, $t_{1/2}$, of 10,7 and 7,5 hours were

calculated respectively for **SucIleCsa** and **GltValCsa**. As can be seen in Figure 3, after 18 hours, the gel was transformed into a clear solution of the corresponding thiol. Control experiments with compounds without the disulfide moiety such as **1** and **2** in Scheme 1, as expected, revealed that the gels were unaffected by the addition of TCEP.

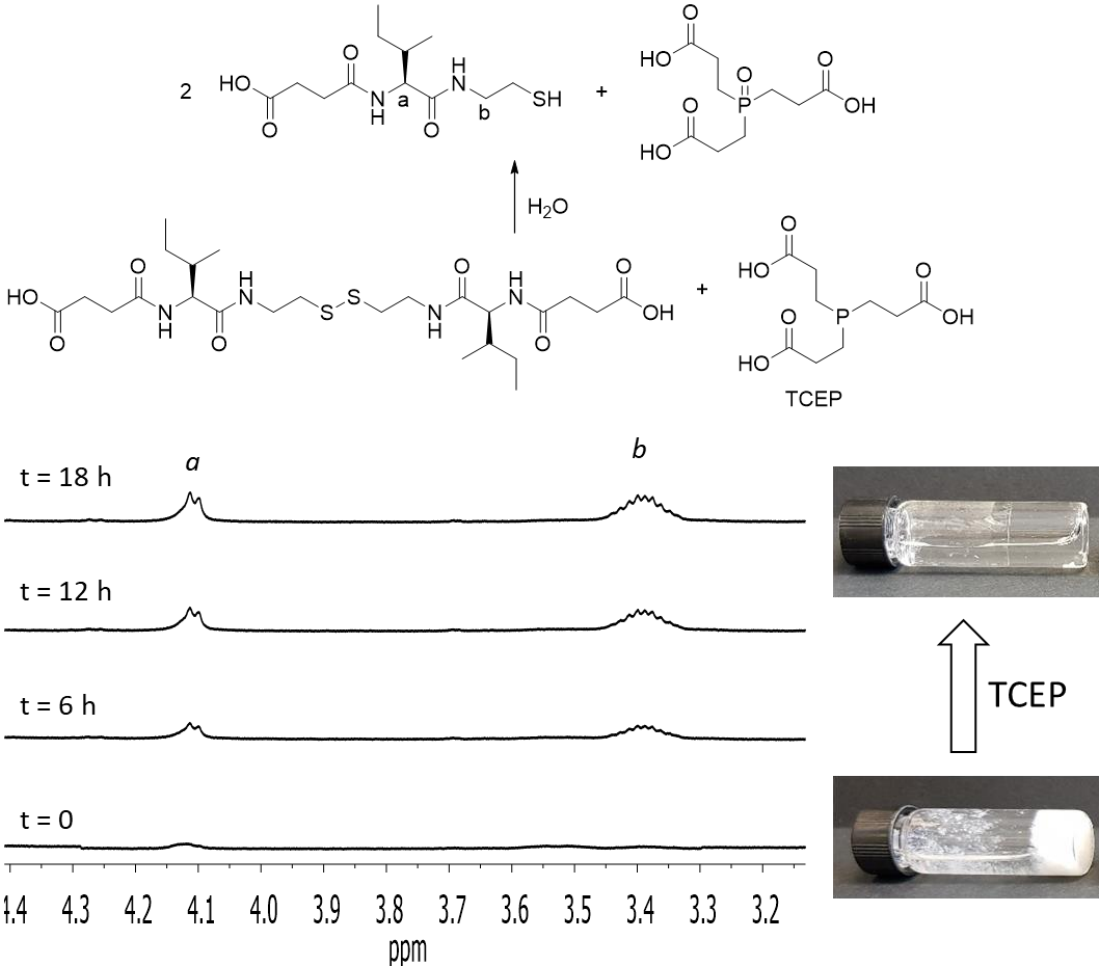


Figure 3. Selected ^{1}H NMR spectra from the kinetic study of **SucIleCsa** gel disassembly in the presence of TCEP.

From an applied point of view, the lability of the molecular hydrogelators could find application for the controlled release of actives. It was envisaged that disulfide bond reduction could potentially be used for the controlled, progressive, discharge of species entrapped in the hydrogels. To test this idea, Bromophenol Blue was loaded in hydrogels of **SucIleCsa** and **GltValCsa**. A solution of TCEP was deposited on top of the gel, and the release of Bromophenol Blue was monitored by UV–Vis at 37.4 ºC, λ_{max}= 437 nm. As shown in Figure

4, the gel of **SucIleCsa** showed a steady release of the dye for 24 h, resulting from progressive gel disassembly. The control experiment in the absence of TCEP showed a partial liberation of Bromophenol Blue, that corresponds to the fraction of the dye that is loosely adsorbed on the fibrillary network and leaches out by diffusion. Similar results were obtained in the case of **GltValCsa** although in this case, the amount of dye release by diffusion out of the fibrillary network in the absence of TCEP was higher (see Figure S10, S11). This behavior is probably the result of the differences in the morphology of the gel networks pointed by electron microscopy, presenting that of **SucIleCsa** a higher aspect ratio and better adsorption capabilities.

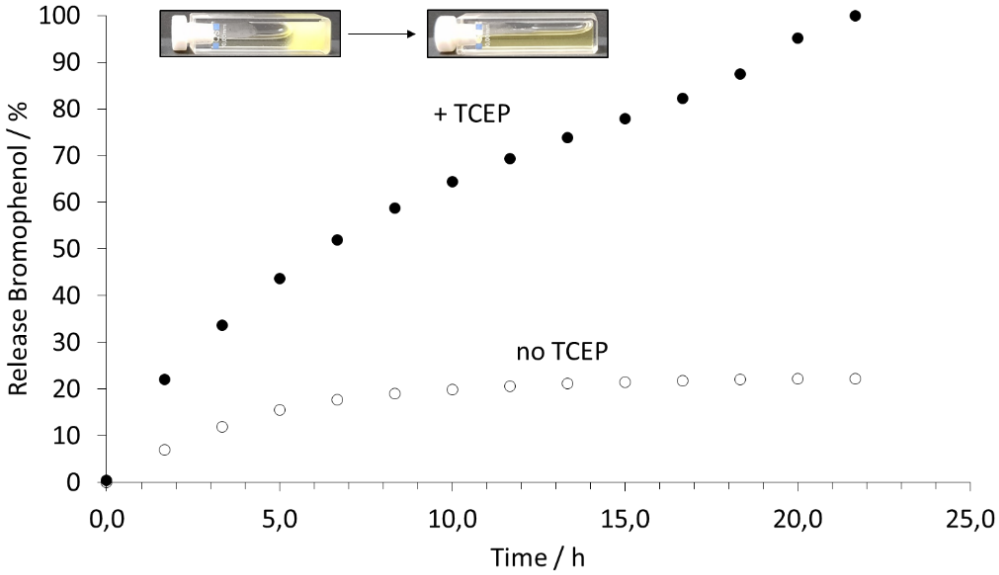
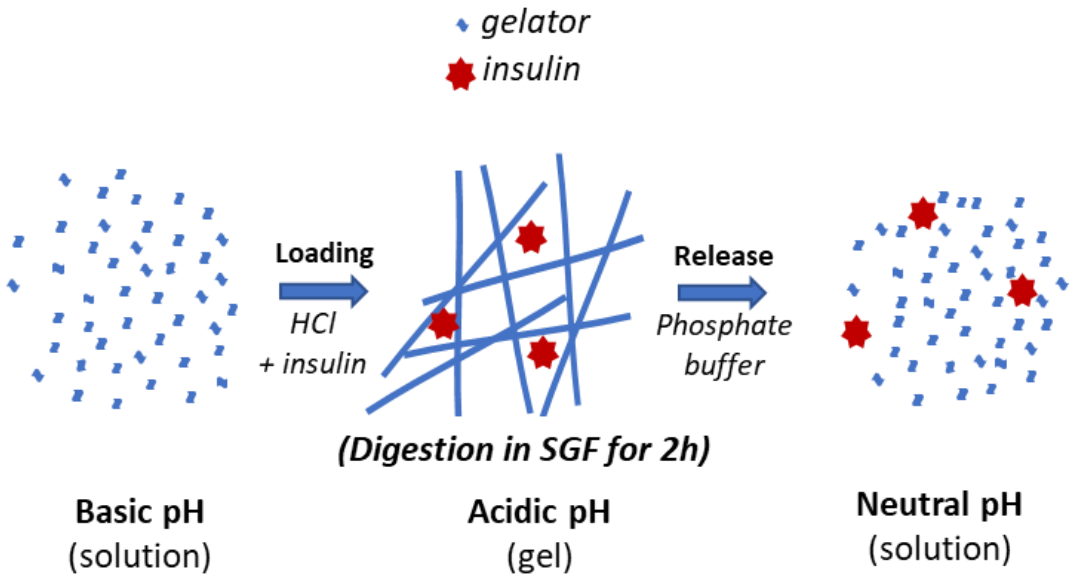


Figure 4. UV–Vis evaluation of the liberation of Bromophenol Blue (0.1 mM) entrapped in the hydrogel formed by **SucIleCsa**.

Finally, the pH responsiveness nature of the gels was exploited for entrapment and later liberation of insulin. Gels were prepared in the presence of insulin (0.25 mg/mL) and incubated in a simulated gastric fluid (SGF) containing pepsin. Under these conditions, free insulin would be hydrolyzed rapidly, precluding its oral administration. The system was incubated for 2 hours, and then pH 7.4 phosphate buffer was deposited on the top of the gel, provoking its disassembly to yield solutions (see Scheme 2). Noteworthy, the pH is rapidly changed from highly acid in the stomach to pH 6.4-7.4 in different areas of the intestine.[61]



Scheme 2. Pictorial representation of entrapment of insulin in a molecular gel and its pH change promoted liberation.

As can be observed in Figure 5, left, notable differences in the gel solubilization kinetics were noted, being the gel formed by **GltValCsa** fully solubilized after 30 min while that of **SucIleCsa** required more than 2 hours for its complete disaggregation (see NMR study in Figure S8, S9). An enzyme-linked immunosorbent assay (ELISA), using colorimetric detection of insulin, revealed that after gel disaggregation at pH 7.4 insulin was recovered quantitatively from gel protected insulin samples. On the other hand, no insulin was detected for control samples because of its hydrolysis (Figure 5, right).

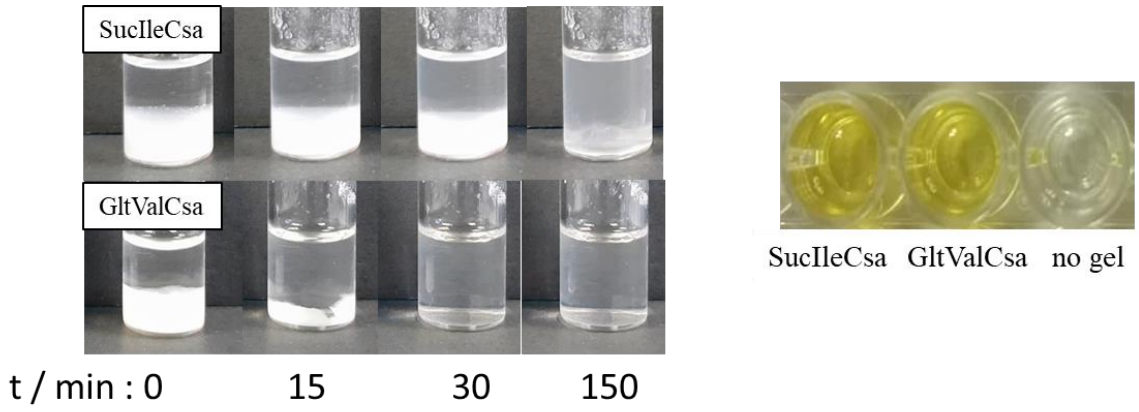


Figure 5. Left: images of insulin-loaded hydrogels in the presence of 0.2 M phosphate buffer (pH 7.4) at different time intervals. Right: wells used in the ELISA evaluation of insulin concentration. Yellow color indicates the presence of unaltered insulin.

3. Conclusions

The compounds **SucIleCsa** and **GltValCsa** form molecular hydrogels in acidic media which are constituted by fibrillar networks visualized by electron and confocal microscopy. Despite being **SucIleCsa** and **GltValCsa** isomers, their fibrillar network morphology differs notably, being that present in the gels of **GltValCsa** formed by flat and straight fibers and that of **SucIleCsa** containing curved and thinner fibers. The rationalization of these differences is far from trivial nowadays, being a significant challenge the prediction of aggregate morphology based on the structure of the molecule involved.

The studied gelators present two stimuli-responsive groups, disulfide, and carboxylic acid. The presence of a reducing agent capable of breaking disulfide bonds into thiol units, such as TCEP, provokes gel disassembly and subsequent release of the entrapped dye, Bromophenol Blue, which is used to prove the validity of the stimuli-driven release system devised. The presence of the carboxylic acid moiety in the gelators limits the pH range of hydrogel existence to acidic pH. For pH values higher than ca. 4 the gelators are ionized and soluble. This property precludes the use of bioactive reduction molecules such as glutathione for gel disassembly, whose reducing characteristics are halted at acidic pH. The present work highlights how the structural motif used in the preparation of the gelators, a cystamine core coupled to amino acids provides the desired gelation and stimuli responsiveness. For application in biological media, the substitution of a carboxylic acid by other functionalities that permit gel formation at neutral pH should be designed in future work.

On the other hand, the stability of the gels in acidic media and their smooth disassembly at neutral pH permits its use for insulin protection. The insulin loaded into the gels resist degradation in the presence of simulated gastric fluid containing pepsin, a medium that hydrolyzes almost instantly free insulin. The fibrillar network of the gel protects insulin from pepsin which, due to its macromolecular nature, probably diffuses very slowly into the gel. Changing pH from acidic to 7.4, as occurs in the gastrointestinal tract, results in gelator

solubilization and insulin release. ELISA analysis shows that insulin released from the gel is recovered quantitatively, while in the absence of gel is completely hydrolyzed. Therefore, the described hydrogelators show successful results in the proof of concept experiment of protection and release of insulin, overcoming the primary barrier for oral administration of drugs, the harsh conditions of pH and presence of hydrolytic enzymes along the gastrointestinal tract.[62]

Overall, relatively simple molecules, which can be prepared quickly on grams scale, form hydrogels which show the sought stimuli responsiveness. The modular nature of their skeleton paves the way for improved properties based on different amino acid building blocks and terminal units.

4. Experimental section

4.1 General considerations

^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent VNMR System spectrometer (500 MHz for ^{1}H NMR, 125 MHz ^{13}C NMR) or Bruker Avance III HD spectrometers (400 MHz and 300 MHz for ^{1}H NMR, 101 MHz and 75 MHz for ^{13}C NMR) in the indicated solvent at 30 ºC. Reactions which required an inert atmosphere were carried out under N_2. Commercially available reagents and *HPLC* grade solvents were used as received. Mass spectra were run by the electro-spray mode (ESMS). Masses spectra were recorded at Mass Spectrometry triple Quadrupole Q-TOF Premier (Waters) with simultaneous Electrospray and APCI Probe. Rheology studies were done on a Discovery HR-1 (TA Instrument) system, 40 mm parallel plates were used during the experiment at the gap of 500 μm. The gels were characterized by the dynamic frequency sweep test in the region of 1-50 Hz with 0.1% strain at 25 ºC. Before the dynamic sweep, a dynamic strain sweep was performed to find out the linear viscoelastic region. The UV/Vis absorption measurements were recorded on a JASCO V-630 spectrophotometer. The measurements were carried out using 4 mL SUPRASIL quartz cells

with 10 mm light path from Hellma Analytics. Transmission electron microscopy (TEM) was performed using a JEOL 2100 microscope equipped with a camera CCD (11 MP). The corresponding fresh gels were applied directly onto a 200 mesh carbon-coated copper grids and stained with one drop of phosphotungstic acid 1 % for 1 min. Excess of solvent and stain was carefully removed by capillary action. Confocal laser scanning microscopy (CLSM) was performed using an inverted confocal microscope Leica TCS SP8. The corresponding fresh gels were stained with Nile red (10 uM) and loaded onto a sterilized Ibidi μ-Slide 8 Well Glass Bottom: # 1.5H (170 mm +/- 5 mm) Schott glass. Excitation of samples was done with a diode laser (514 nm), and images were captured at 63x magnification with HCxPL APO 40.0 x 1.32 oil objective.

4.2 Synthesis of SucIleCsa and GltValCsa

See synthetic procedures and characterization in SI.

4.3 Hydrogel formation

In a representative example, 10 mg of the hydrogelator were placed in a cylindrical glass vial (diameter = 1.5 cm) and dissolved with 500 μL of aqueous NaOH 0.1 M. Then 600 μL of aqueous HCl 0.1 M were added, and the closed vial was allowed to stand at room temperature until the formation of gel (ca. 10 min).

4.4 Gel disassembly with TCEP

1.1 mL of the hydrogels with a concentration of gelator of 18 mM were prepared as described above. Then, 1 mL of aqueous TCEP 40 mM was deposited over the gel, and the closed vial was let to stand at 37.4 °C in a thermostated bath overnight. At this point gel disassembly into a solution was observed. Water was removed by lyophilization, and the residue was dissolved in DMSO-d_6 to obtain its ^{1}H NMR spectrum (see Figure S2, S3).

For the kinetic study of the gelator reduction with TCEP, the gels (18 mM, 550 mL) were mixed (vortex) with 500 mL of 40 mM TCEP solution and then sonicated for 1 min to remove air

bubbles. The mixture was placed inside an NMR tube, and ^{1}H NMR spectra were recorded at 1 h intervals at 37º C (see Figures S4, S5).

Release of Bromophenol blue was studied preparing 1.1 mL of the hydrogels (18 mM) in the presence of Bromophenol Blue (0.1 mM) in a UV-Vis spectroscopy cuvette (1 cm optical path). Then, 1 mL of a 40 mM solution of TCEP (2 eq.) in water was placed over the gels, and the cuvette was sealed and allocated inside the spectrophotometer at 37 ºC. The variation of absorbance with time was monitored at 437 nm. A control experiment with distilled water, instead of the 40 mM TCEP solution, was carried out.

4.5 Gel disassembly triggered by the change of pH

1 mL of the hydrogels with a concentration of gelator of 18 mM were prepared as described above. Then, 1 mL of 0.2 M phosphate buffer pH 7.4 was deposited over the gel, and the closed vial was let to stand at room temperature. After several minutes (see Figure 5) the hydrogel network is completely disrupted what yield a clear solution.

For the NMR study of pH-triggered disassembly, 250 μL of hydrogels of the **SucIleCsa** and **GltValCsa** (18 mM for both compounds) were prepared inside an NMR tube. Then, 250 μL of 0.2 M phosphate buffer pH 7.4 were added. The tubes were sealed and allocated inside the equipment at 30 °C. The successive ^{1}H NMR spectra were recorded at intervals of 10 minutes. All solutions were prepared in D_2O.

4.6 Insulin loading and release

All the experiments were carried out at 37 ºC. Hydrogels (1mL, 18 mM) were made in the presence of human recombinant insulin (0.25 mg/mL). Then, 1 mL of simulated gastric fluid (SGF; 0.2 % w/w sodium chloride and 0.32 % w/w pepsin in 0.08 M aqueous HCl, pH 1.2) was placed over the gel. After 2 h, the supernatant SGF solution was withdrawn and then, 1 mL of 0.2 M phosphate buffer, pH 7.4, was added. After several minutes (see Figure 5) the hydrogel network was utterly disrupted. Control experiments were carried out analogously for aqueous solutions of insulin in the absence of hydrogel. To check the release of undamaged insulin,

duplicated ELISA tests were performed with Human Insulin ELISA Kit (RAB0327, Sigma), monitoring color appearance with a Labtech LT-4000 microplate reader at a wavelength of 450 nm.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
The authors acknowledge financial support from Ministerio de Economía y Competitividad of Spain (grant CTQ2015-71004-R) and Universitat Jaume I (grants UJI-B2018-54; UJI-B2018-30).

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

References

[1] M. A. C. Stuart, W. T. S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov, S. Minko, *Nat. Mater.* **2010**, *9*, 101.

[2] G. Pasparakis, M. Vamvakaki, *Polym. Chem.* **2011**, *2*, 1234-1248.

[3] T. Tanaka, I. Nishio, S. T. Sun, S. Ueno-Nishio, *Science* **1982**, *218*, 467-469.

[4] A. Suzuki, T. Tanaka, *Nature* **1990**, *346*, 345-347.

[5] Y. Osada, H. Okuzaki, H. Hori, *Nature* **1992**, *355*, 242-244.

[6] R. Yoshida, K. Uchida, Y. Kaneko, K. Sakai, A. Kikuchi, Y. Sakurai, T. Okano, *Nature* **1995**, *374*, 240-242.

[7] L. E. Bromberg, E. S. Ron, *Adv. Drug Deliv. Rev.* **1998**, *31*, 197-221.

[8] S. Chaterji, I. K. Kwon, K. Park, *Prog. Polym. Sci.* **2007**, *32*, 1083-1122.

[9] D. Gu, A. J. O'Connor, G. G.H. Qiao, K. Ladewig, *Expert Opin. Drug Deliv.* **2017**, *14*, 879-895.

[10] R. Narayanaswamy, V. P. Torchilin, *Molecules* **2019**, *24*, 603.

[11] P. K. Bolla, V. A. Rodriguez, R. S. Kalhapure, C. S. Kolli, S. Andrews, J. Renukuntla, *J. Drug Deliv. Sci. Technol.* **2018**, *46*, 416-435.

[12] R. G. Weiss, *J. Am. Chem. Soc.* **2014**, *136*, 7519-7530.

[13] E. R. Draper, D. J. Adams, *Chem* **2017**, *3*, 390-410.

[14] D. B. Amabilino, D. K. Smith, J. W. Steed, *Chem. Soc. Rev.* **2017**, *46*, 2404-2420.

[15] X. Du, J. Zhou, J. Shi, B. Xu, *Chem. Rev.* **2015**, *115*, 13165-13307.

[16] M. J. Webber, P. Y. W. Dankers, *Macromol. Biosci.* **2019**, *19*, 1800452.

[17] C. D. Jones, J. W. Steed, *Chem. Soc. Rev.* **2016**, *45*, 6546-6596.

[18] M. Dolores Segarra-Maset, V. J. Nebot, J. F. Miravet, B. Escuder, *Chem. Soc. Rev.* **2013**, *42*, 7086-7098.

[19] Z. Sun, Q. Huang, T. He, Z. Li, Y. Zhang, L. Yi, *ChemPhysChem* **2014**, *15*, 2421-2430.

[20] J. Mayr, C. Saldías, D. Díaz Díaz, *Chem. Soc. Rev.* **2018**, *47*, 1484-1515.

[21] J. Hoque, N. Sangaj, S. Varghese, *Macromol. Biosci.* **2019**, *19*, 1800259.

[22] C. Tang, A. M. Smith, R. F. Collins, R. V. Ulijn, A. Saiani, *Langmuir* **2009**, *25*, 9447-9453.

[23] D. J. Adams, M. F. Butler, W. J. Frith, M. Kirkland, L. Mullen, P. Sanderson, *Soft Matter* **2009**, *5*, 1856-1862.

[24] C. A. Angulo-Pachón, J. F. Miravet, *Chem. Commun.* **2016**, *52*, 5398-5401.

[25] B. Verdejo, F. Rodríguez-Llansola, B. Escuder, J. F. Miravet, P. Ballester, *Chem. Commun.* **2011**, *47*, 2017-2019.

[26] S. C. Lange, J. Unsleber, P. Drücker, H. J. Galla, M. P. Waller, B. J. Ravoo, *Org. Biomol. Chem.* **2015**, *13*, 561-569.

[27] I. S. Okafor, G. Wang, *Carbohydr. Res.* **2017**, *451*, 81-94.

[28] W. Z. Wang, C. Gao, Q. Zhang, X. H. Ye, D. H. Qu, *Chem. Asian J.* **2017**, *12*, 410-414.

[29] M. Externbrink, S. Riebe, C. Schmuck, J. Voskuhl, *Soft Matter* **2018**, *14*, 6166-6170.

[30] K. H. Sun, Y. S. Sohn, B. Jeong, *Biomacromolecules* **2006**, *7*, 2871-2877.

[31] J. H. Ryu, R. T. Chacko, S. Jiwpanich, S. Bickerton, R. P. Babu, S. Thayumanavan, *J. Am. Chem. Soc.* **2010**, *132*, 17227-17235.

[32] X. Zhang, K. Achazi, D. Steinhilber, F. Kratz, J. Dernedde, R. Haag, *J. Control. Release* **2014**, *174*, 209-216.

[33] X. Cai, C. Dong, H. Dong, G. Wang, G. M. Pauletti, X. Pan, H. Wen, I. Mehl, Y. Li, D. Shi, *Biomacromolecules* **2012**, *13*, 1024-1034.

[34] N. Feng, M. Yang, X. Feng, Y. Wang, F. Chang, J. Ding, *ACS Biomater. Sci. Eng.* **2018**, *4*, 4154-4162.

[35] R. A. Gortner, W. F. Hoffman, *J. Am. Chem. Soc.* **1921**, *43*, 2199-2202.

[36] F. M. Menger, K. L. Caran, *J. Am. Chem. Soc.* **2000**, *122*, 11679-11691.

[37] J. P. Wojciechowski, A. D. Martin, P. Thordarson, *J. Am. Chem. Soc.* **2018**, *140*, 2869-2874.

[38] C. J. Bowerman, B. L. Nilsson, *J. Am. Chem. Soc.* **2010**, *132*, 9526-9527.

[39] J. W. Sadownik, R. V. Ulijn, *Chem. Commun.* **2010**, *46*, 3481-3483.

[40] J. Chen, W. Wu, A. J. McNeil, *Chem. Commun.* **2012**, *48*, 7310-7312.

[41] S. Liu, A. Tang, M. Xie, Y. Zhao, J. Jiang, G. Liang, *Angew. Chem. Int. Ed.* **2015**, *54*, 3639-3642.

[42] D. B. Rasale, I. Maity, M. Konda, A. K. Das, *Chem. Commun.* **2013**, *49*, 4815-4817.

[43] Y. Ge, H. Gong, J. Shang, L. Jin, T. Pan, Q. Zhang, S. Dong, Y. Wang, Z. Qi, *Macromol. Rapid Commun.* **2019**.

[44] Y. Gao, J. Lu, J. Wu, J. Hu, Y. Ju, *RSC Advances* **2014**, *4*, 63539-63543.

[45] G. Chen, J. Li, Y. Cai, J. Zhan, J. Gao, M. Song, Y. Shi, Z. Yang, *Sci. Rep.* **2017**, *7*.

[46] L. Milanesi, C. A. Hunter, N. Tzokova, J. P. Waltho, S. Tomas, *Chem. Eur. J.* **2011**, *17*, 9753-9761.

[47] J. Yu, H. Fan, J. Huang, J. Chen, *Soft Matter* **2011**, *7*, 7386-7394.

[48] D. B. Rasale, I. Maity, A. K. Das, *Chem. Commun.* **2014**, *50*, 11397-11400.

[49] T. Yamagata, M. Morishita, N. J. Kavimandan, K. Nakamura, Y. Fukuoka, K. Takayama, N. A. Peppas, *J. Control. Release* **2006**, *112*, 343-349.

[50] X. Gao, Y. Cao, X. Song, Z. Zhang, X. Zhuang, C. He, X. Chen, *Macromol. Biosci.* **2014**, *14*, 565-575.

[51] S. Cikrikci, B. Mert, M. H. Oztop, *J. Agric. Food. Chem.* **2018**, *66*, 11784-11796.

[52] X. Qi, Y. Yuan, J. Zhang, J. W. M. Bulte, W. Dong, *J. Agric. Food. Chem.* **2018**, *66*, 10479-10489.

[53] C. Damgé, C. P. Reis, P. Maincent, *Expert Opin. Drug Deliv.* **2008**, *5*, 45-68.

[54] L. Liu, Y. Zhang, S. Yu, Z. Yang, C. He, X. Chen, *ACS Biomater. Sci. Eng.* **2018**, *4*, 2889-2902.

[55] L. Liu, Y. Zhang, S. Yu, Z. Zhang, C. He, X. Chen, *Biomacromolecules* **2018**, *19*, 2123-2136.

[56] M. Fontanillo, C. A. Angulo-Pachón, B. Escuder, J. F. Miravet, *J. Colloid Interface Sci.* **2013**, *412*, 65-71.

[57] C. A. Angulo-Pachon, J. F. Miravet, *Chem. Commun.* **2016**, *52*, 5398-5401.

[58] C. A. Angulo-Pachón, D. Navarro-Barreda, C. M. Rueda, F. Galindo, J. F. Miravet, *J. Colloid Interface Sci.* **2017**, *505*, 1111-1117.

[59] M. Tena-Solsona, B. Escuder, J. F. Miravet, V. Casttelleto, I. W. Hamley, A. Dehsorkhi, *Chem. Mater.* **2015**, *27*, 3358-3365.

[60] B. Escuder, M. Llusar, J. F. Miravet, *J. Org. Chem.* **2006**, *71*, 7747-7752.

[61] G. Pye, D. F. Evans, S. Ledingham, J. D. Hardcastle, *Gut* **1990**, *31*, 1355-1357.

[62] B. Homayun, X. Lin, H. J. Choi, *Pharmaceutics* **2019**, *11*.

The table of contents entry should be 50–60 words long and should be written in the present tense and impersonal style (i.e., avoid we). The text should be different from the abstract text.

Keyword supramolecular gels

D. Navarro-Barreda, C. A. Angulo-Pachón, B. Bedrina, F. Galindo and J. F. Miravet

A dual stimuli responsive supramolecular gel provides insulin hydrolysis protection and redox-controlled release of actives

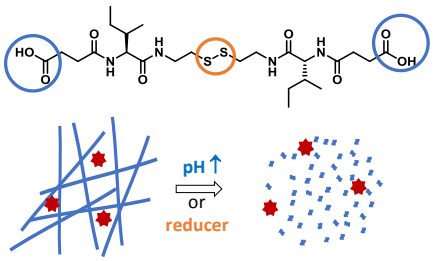


Table of contents entry: Supramolecular hydrogels are prepared containing disulfide units which provide with redox responsiveness to these soft materials. Additionally, the gels can be disassembled at neutral or basic pH values. These properties are used for the release of an entrapped dye in the presence of reductive environment and for insulin hydrolysis protection and its posterior pH-triggered release.